Tuexenia 30: 457–466. Göttingen 2010.

# Population structure of *Pulsatilla patens* in relation to the habitat quality

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## Abstract

This paper presents a study on a population of *Pulsatilla patens* conducted in 2009 in the Natura 2000 wildlife refuge called "Grasslands in the Military Training Grounds in Orzysz" in the Masurian Lake District (NE Poland). The purpose of the study was to determine the population structure of *Pulsatilla patens*, especially with regard to abundance, density, and percentage of life cycle stages in the total population. Correlations were examined between these population features and selected environmental characteristics including cover of phanerogams, cryptogams, litter, and bare soil. For the evaluation of the linear relationship between the variables, Spearman's correlation coefficient was used. In order to identify the factors that have significant influence on the values of the dependent variables, a multiple regression analysis was performed. In total, 316 individuals of *Pulsatilla patens* were recorded: 62 flowering adults, 202 vegetative adults and 52 juveniles. The number of flowers per individual varied from one to 12, the average being three flowers. Most individuals occurred in dry heath, and the smallest number of individuals was found in pine forest. The mean density per habitat ranged from one to 11 individuals per 4 m$^2$. Increased cover of phanerogams and cryptogams caused a decrease in the density of *Pulsatilla patens* individuals.

### Zusammenfassung: Populationstruktur von *Pulsatilla patens* in Abhängigkeit von der Habitatqualität

Diese Veröffentlichung stellt die Forschungsergebnisse zur Population der Finger-Kuhschelle (*Pulsatilla patens*) dar, die 2009 in dem Natura-2000-Gebiet „Graslandgesellschaften auf dem Truppenübungsplatz Orzysz" auf der Masurischen Seenplatte (NE Poland) durchgeführt wurden. Die Forschungen hatten zum Ziel, die Struktur der Population von *Pulsatilla patens* unter Berücksichtigung der Menge, der Dichte und des Anteils von Individuen in verschiedenen Lebenszyklusphasen an der Population festzustellen. Erforscht wurden die Zusammenhänge zwischen den zuvor genannten Populationsmerkmalen und der Deckung von Phanerogamen, Kryptogamen, Streu und offenen Boden. Für die Auswertung der linearen Abhängigkeit zwischen den Variablen wurde der Korrelationskoeffizient nach Spearman angewandt. Zwecks Erkennung von Merkmalen, die den Wert der abhängigen Variablen wesentlich beeinflussen, wurde eine multiple Regressionsanalyse durchgeführt. Festgestellt wurden 316 Individuen von *Pulsatilla patens*, davon 62 blühende und 202 vegetative Exemplare sowie 52 Juvenilpflanzen. Die Blütenzahl je blühender Pflanze betrug zwischen 1 und 12; im Durchschnitt waren es drei Blüten. Die Forschungen ergaben einen negativen Einfluss der Phanerogamen- und Kryptogamendeckung auf die Gesamt- und Juvenilpflanzenzahl.

**Keywords:** age-state class, Masurian Lake District, Northeast Poland, threatened species, vegetation characteristics.

## Introduction

*Pulsatilla patens* (Eastern pasque flower) is a threatened plant species in Europe, listed in the Bern Convention (COUNCIL OF EUROPE 1979) and in Annex II of the European Habitats Directive (EUROPEAN COMMUNITIES 2004). In order to assess the state of preservation of its populations, a monitoring programme was conducted in several countries, e.g. Estonia and Finland (KALLIOVIRTA et al. 2003).

The reasons why populations of the *Pulsatilla patens* are threatened include low competitiveness against other undergrowth plant species, lack of natural disturbances (fires, windblows) in forest ecosystems (UOTILA 1996, KALLIOVIRTA et al. 2006), destruction of flowers and fruit-bearing shoots by animals, hybridization with other species belonging to the genus *Pulsatilla* (UOTILA 1996), reduced seed production due to locally decreasing numbers of pollinating insects (ZYCH 2007), and unfavourable weather conditions such as long and freezing cold winters (CHMURA 2003). Among the major man-made factors threatening the species are destruction and fragmentation of habitats, continuously decreasing forest areas, eutrophication of habitats and cessation of grazing (UOTILA 1996, PILT & KUKK 2002). For small populations near inhabited areas, another serious threat is that people look for these plants to take them into their gardens (WÓJTOWICZ 2000). In Poland, this rare species has been legally protected since 1958. It is included in the Red Data Book of Poland, listed as a low risk (LR) taxon (WÓJTOWICZ 2001). In a recently published Red List of Vascular Plants in Poland (ZARZYCKI & SZELĄG 2006), *P. patens* is considered to be critically endangered. In order to sustain the current population level and the size of the distribution area, a national plan to preserve this species was introduced in 2007 (ZYCH 2007).

In Poland, populations of *P. patens* are disappearing from many sites (CIOSEK 1999, CHMURA 2003, WÓJTOWICZ 2004). A well-preserved population of *Pulsatilla patens* is protected within the site called "Grasslands in the Military Training Grounds in Orzysz", which is part of the Natura 2000 network. The purpose of this study was to determine the current population structure of *P. patens* in this locality, including its abundance, density, and percentage of different life cycle stages. Relationships were estimated between these population characteristics and habitat-related features such as cover of phanerogams, cryptogams, litter, and bare soil.

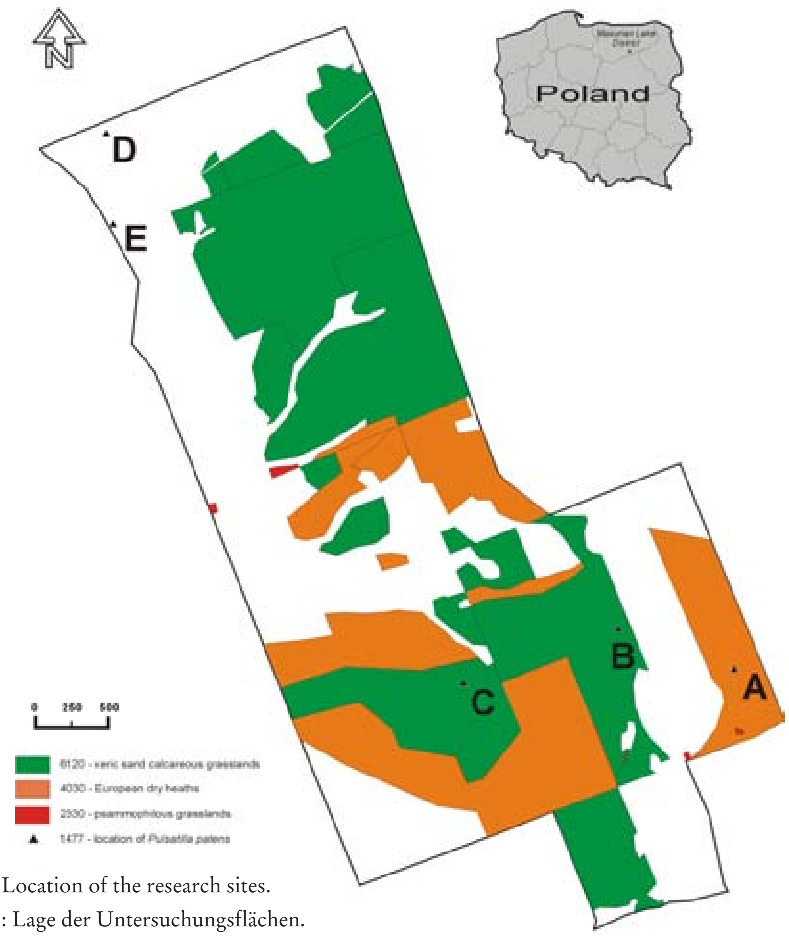


Fig. 1: Location of the research sites.

Abb. 1: Lage der Untersuchungsflächen.

## 2. Study area

The observations were carried out all over the territory of the site called "Grasslands in the Military Training Grounds in Orzysz", which belongs to the Natura 2000 network (Fig. 1). Its size is 1386.23 ha. According to the system of physical and geographical regionalisation (KONDRACKI 2001), this wildlife refuge is situated in NE Poland, in the Masurian Lake District. The landscape of the refuge consists of sandy plains and moraine hills. The vast, open space is overgrown with well-developed xeric sand calcareous grassland and dry heaths. Small areas are covered with pioneer psammophilous grassland on oligotrophic inland sands. There is a small eutrophic lake in the refuge. In the central part there are waterlogged depressions covered with peat bog plants and small alder carrs. The northern part of the area is overgrown with pine forest.

## 3. Material and methods

### 3.1. Study species

*Pulsatilla patens* (L.) Mill. (Fig. 2) is a monoecious, long-lived (tens of years) hemicryptophyte with an upright, branching rhizome, which makes older plants form clumps. Two types of buds are produced annually: one replaces the terminal bud, which is transformed into a flowering apex, and one becomes dormant. The flower bud remains enclosed by the bud scales until the spring (mid-April until mid-May) of the third season. The second type of bud produced annually is smaller and enters a prolonged dormancy after producing protective bud scales. The development of these two types of buds follows a regular pattern. The reserve of viable dormant buds is augmented annually and enables the plant to regenerate new branches, if terminal apices are damaged. The largest, most highly branched individuals, which can form over 20 flowers and 50 leaves in one growing season, are frequently found in locations that are grazed or burned (WILDEMAN & STEEVES 1982). Individuals of *Pulsatilla patens*, especially older ones, are very sensitive to root damage (WÓJTOWICZ 2000). The seeds are dispersed by wind in June and July over short distances. In good conditions (warm and moist weather) germination occurs in late summer, but if the weather is cold and dry, it is delayed until the next spring, or seeds may remain in the transient seed bank (PILT & KUKK 2002). The extent of the formation of leaf rosettes and of flowering

Fig. 2: Eastern pasque flower (*Pulsatilla patens*) in the "Grasslands in the Military Training Grounds in Orzysz" (Photo: Barbara Juśkiewicz-Swaczyna, May 2009).

Abb.2: Finger-Kuhschelle (*Pulsatilla patens*) an ihrem Standort „Rasengesellschaften auf dem Truppenübungsplatz Orzysz“ (Foto: Barbara Juśkiewicz-Swaczyna, Mai 2009).

and fruit bearing shoots depends on weather conditions such as winter temperatures, snow cover, autumn precipitation, temperature, and sunshine duration in spring (Wójtowicz 2000).

*Pulsatilla patens* has a circumpolar distribution from Eurasia to North America (Hulten & Fries 1986). In Europe, it occurs in the central and central-eastern parts, with its northern limit of distribution at 66° northern latitude in Russia (Jalas & Suominen 1989). The western border runs across Germany (Tutin & Akeroyd 1993), although since 1970 all populations except one in the nature reserve „Garchinger Heide“ located 15 km north of Munich (Röder & Kiehl 2006) have become extinct because of changing land use or abandonment (Schönfelder & Bresinsky 1990). In some localities, for example in Finland (Uotila 1969, 1996) or in Estonia (Pilt & Kukk 2002), populations of *Pulsatilla patens* are considered to be relicts. In Asia, the range of *P. patens* covers Siberia, Mongolia and northern China (Wang & Bartholomew 2001). In North America, the species occurs in the central-western USA, in central and north-western Canada and in eastern Alaska (Wildeman & Steeves 1982).

In Poland, most sites of *P. patens* comprising large numbers of individuals (tens to hundreds) are located in the north-eastern part of the country. In central and south-eastern parts of Poland, there are only few, rather evenly scattered sites each of which contains only a few individuals. In western and south-western Poland, *P. patens* is a rare species (Wójtowicz 2001).

*P. patens* is associated with boreal forests of the class *Vaccinio-Piceetea* (Matuszkiewicz 2001). Sporadically, this species occurs in xerothermic and psammophilous grasslands (Ceynowa 1968, Ciosek 1999). Occurrences of *P. patens* have also been recorded in calamine areas in Upper Silesia (Nowak et al. 2000). In other regions, this species occurs in various habitats, including calcareous grasslands in Germany (Röder & Kiehl 2006); open, dry, pine-dominated forests in Finland, mostly on eskers and adjacent sandy areas, pastures, path- and roadsides and at the edges of yard areas (Uotila 1996, Kalliovirta et al. 2006); in steppe and wood-steppe communities in Russia (Rysina 1981); and in pine-dominated boreal heath forests of the *Cladonia* or *Calluna* site type and in dry boreal forests of the *Vaccinium vitis-idaea* site type, occasionally also in more humid *Vaccinium myrtillus* site type habitats in Estonia (Pilt & Kukk, 2002).

### 3.2. Data collection

The population structure of *Pulsatilla patens* was studied in late April and early June 2009. During the field work, three habitat types with occurrences of *Pulsatilla patens* were distinguished: I – dry heath (site A), II – xeric sand calcareous grassland (sites B and C) and III – pine forest (sites D and E). The exact locations of the sites A–E were recorded by GPS coordinates (Table 1). Fourty-nine research plots of 2 m × 2 m were established at these sites. The number of individuals was estimated in each of the plots, and the number of flowers was counted. In each location, all individuals were recorded and classified into life cycle stages: juvenile, vegetative, and flowering individuals. It is somewhat difficult to determine the exact age of individuals belonging to this species, as a group of leaf rosettes or a clump need not always comprise one genetic individual, because several seeds may disperse and establish together. Excavation of plants or DNA analyses are the ways to verify whether a clump consists of one or more individuals (Pilt & Kukk 2002). In my research, samples for genetic analysis were collected, but the results are not yet available. According to Laarmann (2001), the number of leaves per *P. patens* individual is directly related to its age: juvenile 1–3 leaves, vegetative adults more than 3 leaves. This approach has also been adopted by Röder & Kiehl (2006) and by the author of this paper. Kalliovirta et al. (2006), on the other hand, assumed leaf rosettes closer than 10 cm from each other to belong to

Table 1: Overview of the five analysed sites, with geographic coordinate, population and environmental characteristics

Tab. 1: Übersicht der untersuchten Standorte mit geografischen Koordinaten und Kenngrößen zur Populationsstruktur und zu den Umweltbedingungen

| Habitat | Site | Geographic coordinates | Total number of… | | | | | | Mean density of individuals per 4 $m^2$ | Mean cover per 4 $m^2$ (%) of… | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | plots | individuals | flowering individuals | vegetative individuals | juvenile individuals | flowers | | phanerogams | cryptogams | litter | bare soil |
| I | A | 53.44140° N, 21.59666° E | 43 | 277 | 52 | 181 | 44 | 147 | 6.4 | 63 | 13 | 29 | 5 |
| II | B | 53.44195° N, 21.58949° E | 2 | 18 | 2 | 12 | 4 | 4 | 9.0 | 60 | 12 | 30 | 5 |
| | C | 53.43875° N, 21.57527° E | 2 | 2 | 1 | 1 | 0 | 1 | 1.0 | 78 | 30 | 30 | 10 |
| III | D | 53.46129° N, 21.56211° E | 1 | 8 | 2 | 4 | 2 | 8 | 8.0 | 40 | 30 | 10 | 15 |
| | E | 53.45657° N, 21.56029° E | 1 | 11 | 5 | 4 | 2 | 33 | 11.0 | 30 | 40 | 20 | 10 |
| Total | | | 49 | 316 | 62 | 202 | 52 | 395 | - | - | - | - | - |

the same individual and treated rosettes further apart from each other as separate individuals. In all plots, the total cover of phanerogams, cryptogams, litter, and bare soil were recorded to characterize the habitat quality.

### 3.3. Statistical methods

All data collected at the 49 sites were used for the presentation of the life cycle stages structure of the population and the vegetation cover characteristics. In order to determine the relationships between the density of individuals (total and per life cycle stage) and structural variables (cover of phanerograms, cryptogams, litter, and bare soil), the largest set of data comprising 43 observations in site A of habitat I was used. This was done with Spearman's rank correlations at a significance level of $\alpha = 0.05$. In order to grasp the features that have significant influence on the values of the variables, multiple regression analyses were performed, selecting the best subset of independent variables at a significance level of $\alpha = 0.05$. Four parameters were taken as dependent variables: total number of individuals, number of flowering individuals, number of vegetative individuals, and number of juvenile individuals. The independent variables consisted of cover of phanerogams, cryptogams, litter, and bare soil. All statistical analyses were performed using STATISTICA 8 (Hill & Lewicki 2006).

## 4. Results

In 2009, the *Pulsatilla patens* population at the site "Grasslands in the Military Training Grounds in Orzysz" consisted of 316 individuals. Most of them (202) were vegetative, the fewest (52) juvenile individuals. Flowering individuals (62) formed 395 flowers in total. The number of flowers per individual varied from 1 to 12, the average being 3 flowers (Table 1). Only two individuals had fruits. The observations completed in June 2009 showed that most of the flowers were damaged, most probably by animals. Stalks were found as clear indicators of removed flowers.

The distribution of the individuals over the studied area was uneven – the majority individuals occurred in site A in dry heath (43 plots), and the smallest number of individuals was found in sites D and E in the pine forest (1 plot). The mean density in a plot was between 1 and 11 individuals (Table 1).

The analysis of the ontogenetic structure of the *Pulsatilla patens* population revealed that the proportion of both flowering and juvenile individuals did not differ between habitats (Fig. 3). The proportion of vegetative individuals in habitat I was the same as in habitat II, but it was significantly higher than in habitat III ($p = 0.044$). In all habitats, the highest cover was observed for phanerogams (from 35 to 69%), whereas the cover of cryptogams ranged between 13 and 35%, the cover of litter varied from 5 to 30%, and the cover of bare soil was between 5 and 18% (Fig. 4).

The correlation analysis for site A revealed a significant negative correlation between the total number of individuals and the cover of phanerogams, cover of cryptogams and cover of litter. A positive correlation was determined between the total number of individuals and the cover of bare soil (Table 2).

Table 2: Results of the Spearman rank between the number of individuals of *Pulsatilla patens* per 4 m² and the cover of phanerogams, cryptogams, litter and bare soil for plots in site A ($n = 43$); $r_s$ – Spearman correlation coefficient; $p$ – significance.

Tab. 2: Ergebnisse der Spearman-Korrelation zwischen der Individuenzahl von *Pulsatilla patens* und den Deckungswerten von Phanerogamen, Kryptogamen, Streu und offenem Boden für Standort A ($n = 43$); $r_s$ – Spearman-Korrelationskoeffizienten; $p$ – Signifikanz.

| Characteristic | $r_s$ | $p$ |
|---|---|---|
| Total number of individuals – cover of phanerogams | –0.4498 | 0.01 |
| Total number of individuals – cover of cryptogams | –0.3432 | 0.05 |
| Total number of individuals – cover of litter | –0.3152 | 0.05 |
| Total number of individuals – cover of bare soil | 0.3908 | 0.01 |

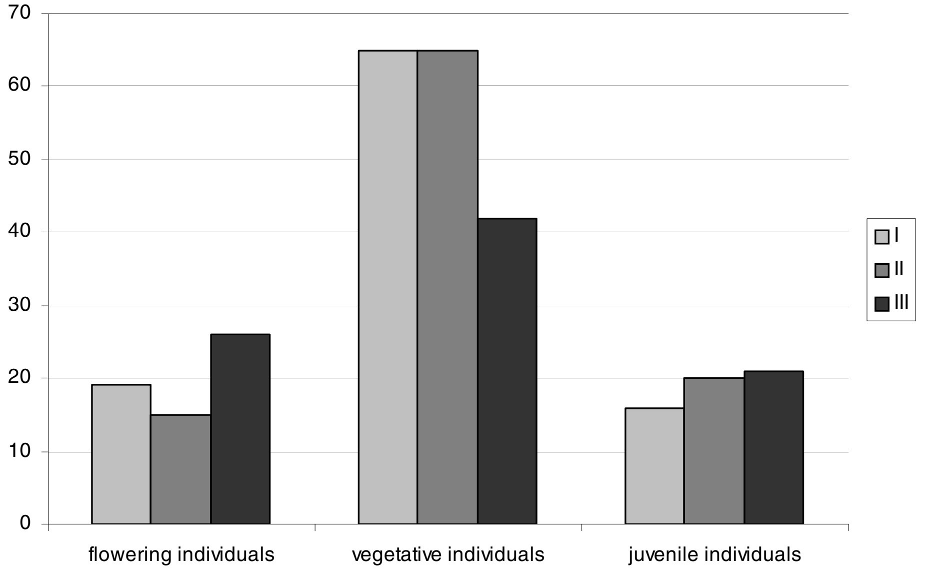


Fig. 3: Life cycle stage structure of the *Pulsatilla patens* population in different habitats (I – dry heath, II – xeric sand calcareous grassland, III – pine forest).

Abb. 3: Populationsstruktur der *Pulsatilla patens*-Population, getrennt nach Habitaten (I – trockene Heide; II – kalkreicher Sandtrockenrasen; III – Kiefernwald).

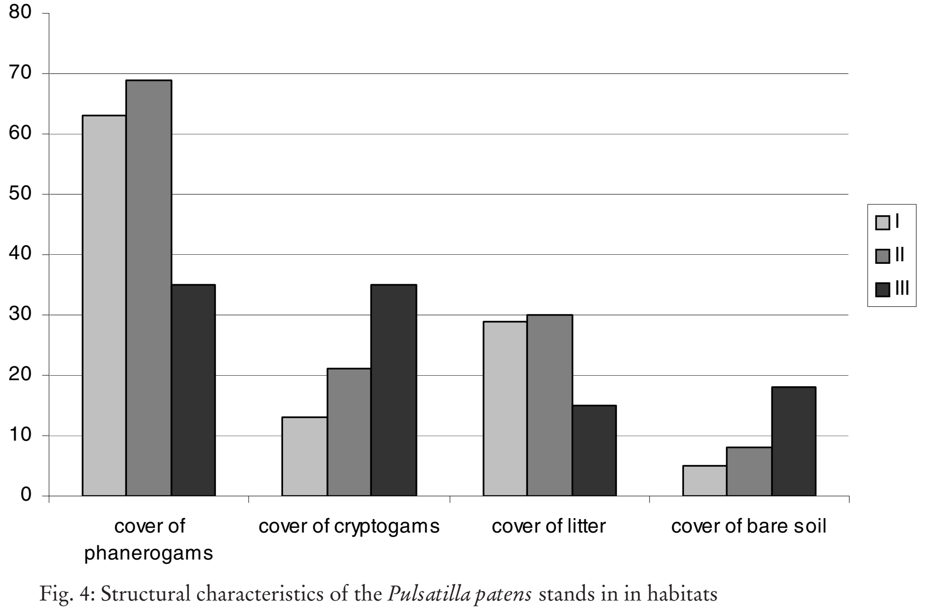


Fig. 4: Structural characteristics of the *Pulsatilla patens* stands in in habitats (I – dry heath, II – xeric sand calcareous grassland, III – pine forest).

Abb. 4: Strukturelle Charakterisierung der Pulsatilla patens-Standorte, getrennt nach Habitaten (I – trockene Heide; II – kalkreicher Sandtrockenrasen; III – Kiefernwald).

Table 3: Statistically best subset of variables explaining the dependent variable $y$ ($y = a + b\,x_1 + c\,x_2 + d\,x_3$); $a$–$d$ – regression coefficients; $x_1$, $x_2$, $x_3$ – independent variables; $p$ – significance; $R^2$ – explained variance.

Tab. 3: Statistisch beste Variablenteilmenge, die die abhängige Variable $y$ erklärt ($y = a + b\,x_1 + c\,x_2 + d\,x_3$); $a$–$d$ – Regressionskoeffizienten; $x_1$, $x_2$ $x_3$ – unabhängige Variablen; $p$ – Signifikanz; $R^2$ – erklärte Varianz.

| $y$ | $a$ | Cover of phanerogams ($x_1$) $b$ | Cover of cryptogams ($x_2$) $c$ | Cover of litter ($x_3$) $d$ | $p$ | $R^2$ |
|---|---|---|---|---|---|---|
| Total number of individuals | 21.3052 | –0.1587 | –0.1000 | – | <0.001 | 0.4127 |
| Number of vegetative individuals | 15.9045 | –0.1284 | –0.1014 | – | <0.001 | 0.4053 |
| Number of juvenile individuals | 3.2416 | – | – | -0.0378 | 0.029 | 0.2139 |

The total number of individuals and the number of vegetative individuals could be explained by a subset of two variables – cover of phanerogams and cover of cryptogams – explaining 41% of the variance in both cases (Table 3). While litter was not included in the variable subset of the model for these two dependent variables, in the case of the number of juvenile individuals it was the only variable in the obtained subset, with 21% of the total variance explained (Table 3). The number of flowering plants could not be explained by any of the independent variables involved in the analysis. In none of the four multiple regressions, the best subset of independent variables contained the cover of bare soil; therefore this variable has not been included in Table 3. The residual analysis revealed a normal distribution of the residuals, which confirms that the set of data had been established correctly.

## 5. Discussion

Considering the biology of the species, monitoring of the Eastern pasque flower requires observations conducted at least twice during the same growing season: during the flowering phase and later, when vegetative individuals appear. The Estonian study (KALLIOVIRTA et al. 2003) showed that the timing of monitoring is crucial to determining the true number of individuals in a population. This condition has been met in my study. The size of *Pulsatilla patens* populations in Poland and in other countries is highly variable – from just a few individuals to hundreds. With this information in mind, the population analysed in the present paper should be regarded as large.

Many authors (CHMURA, 2003, KALLIOVIRTA et al. 2006, RÖDER & KIEHL 2006) evaluate the structure of populations of Eastern pasque flower according to the proportion of individuals in different age-state classes. KALLIOVIRTA et al. (2003) claim that the most useful population parameters to record are the numbers of generative, vegetative, and juvenile individuals. The size of individuals, the number of flowers and seeds are also fairly easy to quantify. Environmental parameters are important to describe, including the amount of open ground and litter, cover of moss and herb layers and the canopy openness at the site. The majority of these parameters were included in my study. In each of the analysed habitats, vegetative individuals were most, juveniles least numerous. Similar relationships were found in the population growing on Sodowa Mount in Jaworzno (CHMURA 2003). The ontogenetic structure of the population near Orzysz was also similar to that of the populations present in ancient grasslands described by RÖDER & KIEHL (2006) in the nature reserve "Garchinger Heide" in Germany. Small proportions of flowering individuals and juveniles have also been noticed by KALLIOVIRTA et al. (2006) in the populations found in Finland. In their previous studies from the same area, KALLIOVIRTA et al. (2003) distinguished three types of populations: (i) evidently increasing, in which vegetative individuals and seedlings prevailed; (ii) stable, in which the proportion of the individuals in various stages remained almost unchanged; and (iii) decreasing, in which vegetative individuals constituted up to 96% of the total population and seedlings were usually completely absent. According to this classification, it can be assumed that the population in Orzysz is an increasing one.

Proper habitat conditions are crucial for the growth and regeneration of populations of the Eastern pasque flower. High light intensity seems to be important for the flowering individuals – the number of fertile individuals and the number of flowers per individual are both highest in open sites. *Pulsatilla patens* shows a preference for places where the layer of moss and litter is disturbed. This seems to be especially important for seedlings, which are not able to establish themselves in a thick moss layer (UOTILA 1969). Such a relation was confirmed by KALLIOVIRTA et al. (2006), although they pointed to the fact that the relationship between habitat and population characteristics needs to be considered in more detail. According to these authors, the highest numbers of juveniles were found on sites with intermediate cover of mosses. This observation suggests that a closed moss layer inhibits seedling establishment, but the complete absence of mosses also has an unfavourable effect. Optimal sites for seedling recruitment are characterized by a heterogeneous fine-scale pattern of bare ground patches and mosses. Also my study of the Orzysz population shows that the highest numbers of juveniles occurred in places where the cover of cryptogams was moderate, between 12 and 13% (see Table 1). Besides, the negative effect of cryptogam cover on the number of vegetative individuals was verified. Statistical analysis proved that the total number of individuals and the number of vegetative individuals was negatively related to the cover of phanerogams and cryptogams.

This relationship has also been mentioned by other authors cited in this paper; in Germany, e.g., RÖDER and KIEHL (2006) found a negative correlation between the number of individuals of all age-state classes and the cover of phanerogams and litter in ancient grasslands areas, whereas in young grasslands, the aforementioned habitat characteristics played a positive role by protecting *P. patens* seedlings.

According to KALAMEES et al. (2005), important factors that affect germination and seedling establishment of *Pulsatilla patens* are cyclic, natural, or controlled wood fires, which change light intensity conditions and destroy moss and litter layers. Additionally, as a result of fires in forests, charcoal is accumulated in the soil, which inactivates phytotoxic phenolic compounds secreted by plants of the family *Ericaceae*. Similar conclusions, based on results of a pot experiment, were formulated by NIITOTS (2007), who stated that forest floor organic matter as a potential source of phenolic compounds is responsible for the poor regeneration of *Pulsatilla patens* in nature. When forest organic matter and charcoal were both added to the soil, plant growth was most intense.

In the present study, emergence and seedling establishment among Eastern pasque flower were not observed due to the lack of fruit-bearing individuals in the described population.

The negative influence of plants belonging to the family *Ericaceae* on populations of other plants, including *Pulsatilla patens*, has been implied by KALAMEES et al. (2005). In the light of my studies, it seems interesting that the pasque flower was most numerous in dry heath, whereas in plots without *Ericaceae* such as xeric and calcareous grassland, only single individuals of this species were found. To explain this finding, further studies on the habitat quality need to be conducted. It would also be interesting to examine the relationship between the presence of *Pulsatilla patens* and the occurrence of other plant species.

It is interesting that the characteristics of the habitats used for multiple regression analysis that remained in the best subset of variables explain only 21 to 41% of changes in the abundance of individuals. This means that other characteristics should be found to complete this subset of characteristics.

**Acknowledgements**

I am deeply grateful to Professor Janusz Terlecki for his help in performing the statistical analysis of the results, his discussion of the results and valuable comments. I would like to thank anonymous reviewers, Monika Janišová and Jürgen Dengler for their valuable remarks on this paper. I would also like to thank Jolanta Idżkowska for translating this paper into English and Aiko Huckauf for the linguistic verification.

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Co-ordinating editor: Monika Janišová
Manuscript received: 25.08.2008; accepted: 01.03.2010